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HIGH FIELD ION MOBILITY METHOD AND APPARATUS FOR DETECTION OF BIOMARKERS

RELATED APPLICATIONS

This application is a continuation-in-part of US Application Serial No. 10/321,822, filed December 16, 2002, entitled "MICROMACHINED FIELD ASYMMETRIC ION MOBILITY FILTER AND DETECTION SYSTEM," by Raanan A. Miller and Erkinjon G. Nazarov, incorporated herein by reference, of US Application Serial No. 10/082,803, filed February 21, 2002, entitled "LONGITUDINAL FIELD DRIVEN FIELD ASYMMETRIC ION MOBILITY FILTER AND DETECTION SYSTEM," by Raanan A. Miller and Markus Zahn, incorporated herein by reference, and of U.S. Application Serial No. 10/187,464, filed 06/28/02, entitled "SYSTEM FOR COLLECTION OF DATA AND IDENTIFICATION OF UNKNOWN ION SPECIES IN AN ELECTRIC FIELD" by Lawrence A. Kaufman, Raanan A. Miller, Erkinjon G. Nazarov, Evgeny Krylov, and Gary Eiceman, incorporated herein by reference, and U.S. Application Serial No. 10/462,206, filed 06/13/03, entitled METHOD AND APPARATUS FOR CONTROL OF MOBILITY-BASED ION SPECIES IDENTIFICATION, by Raanan A. Miller, Erkinjon G. Nazarov, Evgeny Krylov, and Gary A. Eiceman, incorporated herein by reference.

This application claims the benefit of U.S. Provisional Application Serial No. 60/422,534, filed 10/31/02, entitled "HIGH FIELD ION MOBILITY METHOD AND

APPARATUS FOR DETECTION OF BIOMARKERS," incorporated herein by reference. The entire teachings of the above application(s) are incorporated herein by reference.

FIELD OF INVENTION:

This invention relates to detection of bio-markers, and more particularly, to identification of biological materials in a sample by detection of bio-markers using high field ion mobility spectrometry.

BACKGROUND OF THE INVENTION:

Spectrometers are used in chemical analysis for identification of compounds in a sample. These systems may take samples directly from the environment or they may incorporate a front end device to separate or prepare compounds before analysis. In some cases a quick indication of presence of particular compounds in a sample is needed, while at other times the goal is complete identification of all compounds in a chemical mixture.

Notwithstanding an advanced state of the art in chemical detection, the need continues for improved field-portable detectors for airborne biological factors, including detection of bacterial spores. Ion mobility spectrometers have been used to detect various chemical and biological compounds, with some success in field-portable equipment.

There are several species of spectrometers based on ion mobility. These technologies include ion mobility spectrometry (IMS) and high field asymmetric waveform ion mobility spectrometry (FAIMS) systems, among others.

Commercially available IMS systems are based on time-of-flight (TOF-IMS), i.e., they measure the time it takes ions to travel from a shutter-gate to a detector

through an inert atmosphere (1 to 760 Torr.). The drift time is dependent on the mobility of ions in a low electric field based on size, mass and charge, and is characteristic of the ion species detected. TOF-IMS has been used for detection of many compounds including narcotics, explosives, and chemical warfare agents, and at least one TOF-IMS system has been adapted for use in a field-portable device for detection of bacterial spores in the environment.

Sample preparation remains an important part of the detection process. This is particularly true for gas phase analysis of samples that are not easily volatilized. Pyrolysis provides a well-accepted volatilization technique for GC, MS and other types of gas phase analysis. In a known pyrolysis-gas chromatography/ion mobility spectrometer, a particle collector/pyrolizer is used as a front end on a GC/IMS package. The system evaluates a bio-sample by characterization of pyrolysis products; these often are characteristic chemical building blocks of the sampled compounds, and are referred to as "biomarkers".

Favorable results have been obtained from pyrolitic methods for detection of biomarkers. For example, dipicolinic acid (DPA) has been recognized as a biomarker for bacterial spores or sporulating cells. An intermediate pyrolysis product of DPA, namely picolinic (2-pyridinecarboxylic) acid, exhibits good gas chromatographic properties and serves as a highly specific biomarker for sporulating microorganisms. In fact, interfacing of a pyrolysis (Py) module to an existing hand-held gas-chromatography-ion-mobility spectrometry (GC/IMS) device has been employed for detection of picolinic acid as a biomarker of sporulating cells.

However, a known limitation for TOF-IMS is attributed to a diffusion process, wherein some ions of interest will contact the inner walls of the drift tube during their transit toward the collector and will be unintentionally neutralized thereby. These unintentionally neutralized ions of interest will be lost and will not be detected at the collector plate, which degrades sensitivity and detector efficiency. This is of particular concern when positive and reliable detection is required of bio-hazards. This is certainly

true when the real-time need for reliable and accurate detection of toxins, such as chemical warfare agents, even at trace amounts, is considered.

High field FAIMS devices offer an alternative to the low field TOF-IMS ion mobility process. In FAIMS, ion filtering is achieved based on accentuating differences in mobility of ionized molecules ("ions") in a high field. The high field mobility differences are used for signature identification of chemical species in an ionized sample. FAIMS filtering is an efficient process, combining controlled neutralization of unselected ion species while passing selected ion species for detection.

The FAIMS filtering mechanism is based on interaction between ions and the net applied electric field. In a typical FAIMS device, an ionized sample is carried by a transport mechanism, such as a carrier gas, between filter electrodes facing each other across the flow path, forming an analytical gap. A strong periodic asymmetric RF field is generated between these electrodes transverse to the flow of ions in the gap. The FAIMS field alternates between high and low field strengths. These field conditions tend to drive the ions transversely into the filter electrodes, causing their intentional neutralization.

However, a DC compensation is also applied to the filter electrodes with the result that the transverse motion for a particular ion species will be minimized, and this selected ion species will be passed through the filter for detection. The passed ions are collected at a detector, such as a charge collector electrode. When the ions impact the electrode they transfer their charge to the electrode and the resulting current is read by an electrometer. The electric field conditions required to permit a particular ion to penetrate though the filter to the detector are specific to each ion species. By noting the applied field conditions and compensation voltage and the current at the detector electrode, and by comparison to a lookup table of data for known species, the detected ions species can be identified.

Ideally, a full spectrum of an ionized sample should be obtained by scanning the compensation voltage during analysis. In this manner, various compounds in a complex mixture can be detected in a single scan. Detection intensity peaks are associated with field and compensation values, and this data is matched with stored detection data, to enable identification of the detected species.

These FAIMS devices have concentric-cylindrical electrodes that achieve a focusing effect between the electrodes, causing certain species of ions to focus in the

flow path while other ion species will be disbursed and neutralized by contact with the cylinder walls.

A class of known prior art FAIMS devices is described as having concentric cylindrical electrodes. In these known devices, ions of one polarity are transported in an analytical gap defined between facing surfaces of outer and inner concentric cylindrical electrodes. Such cylindrical electrodes for a drift tube that is long enough as to enable the filter fields to produce an ion focusing effect in the analytical gap. This focusing favors ions having high mobility in the FAIMS high field. Thus ions with high mobility tend to focus in the ion flow between these cylindrical electrodes, which assists in FAIMS filtering and delivery of this class of ions. However, ions with lower mobility do not benefit from this focusing effect and tend to disperse and be neutralized in such device, which results in lower detection efficiency for those ions. Hence, a conventional concentric cylindrical FAIMS device is best adapted to detection of biomaterials having ionized biomarkers that have high ion mobility parameters.

Furthermore, ions with different mobility will have different coefficients of transition through the analytical gap of a conventional concentric cylindrical device. Therefore a calibration burden must be met to obtain accurate quantitative detection results for such device.

Still further, such devices ordinarily are capable of detecting only positive or negative ions in any one cycle, and therefore must run a series of detection cycles to obtain a detection profile including both positive and negative ions derived from a given ionized sample.

In view of the foregoing, and in the light of present bio-threats, there is a strong and continuing interest in improved approaches to bio-sample characterization, particularly as may be provided in compact and portable devices.

It is therefore an object of the present invention to provide a functional, sensitive and reliable spectrometer that overcomes the limitations of the prior art.

It is another object of the present invention to provide a functional, small, field-

portable spectrometer that can discriminate between and can identify biological materials based on differential ion mobility in a high electric field.

It is a further object of the present invention to provide a chemical sensor that is able to operate rapidly, affording real-time or near real-time detection, and achieving chemical species identifications with a high level of confidence.

SUMMARY OF THE INVENTION:

Practices of the present invention are directed to method and apparatus for detection and identification of biologically significant compounds. Biological samples yield bio-chemical marker products which are isolated, detected and identified. Practices of the invention are sensitive to parts per billion and parts per trillion. Illustrative practices of the invention include detection of spores (anti-terrorism), decaying flesh (food quality), human breath (breathalyzer detector), and urine (urinalysis), for example.

Embodiments of the invention detect and identify species of ions representing ionized biomarkers that are derivatives of the detected biological materials. This biomarker detection and identification process is based on high field differential ion mobility techniques performed in favorable conditions provided by apparatus of the invention.

The disclosed innovation enables rapid detection and identification of bio-compounds, including compounds that cannot be resolved by other analytical techniques. Such detection and identification can be made rapidly and with a high level of confidence. Practice of the invention is sensitive to parts per billion and even parts per trillion levels.

The present invention is an improvement of prior art FAIMS technology. Devices of the present invention enable differential mobility discrimination of ions species based on the effect on ions as they travel in a transverse asymmetric high field generated between the filter electrodes of the system. The FAIMS systems of the

invention are also known as high field differential mobility spectrometers (DMS), although the term FAIMS is used herein.

The present invention may be practiced with various FAIMS configurations. Preferred embodiments feature compact and field-portable, wide-spectrum, dual mode, FAIMS systems, such as taught in US Patents 6,495,823 and 6,512,224 and in copending U.S. Application Serial No. 10/187464, filed 06/28/02, and U.S. Application Serial No. 10/462206, filed 06/13/03, all incorporated herein by reference.

FAIMS devices of the invention may incorporate various electrode configurations, including non-cylindrical, plate, parallel, planar, flat, and/or non-focusing, for example. A preferred practice of the present invention is generally referred to as "plate-type", although it will be understood embodiments may use facing electrode portions, segments, sections, or plates. Thus various electrode configuration are possible other than the concentric cylindrical electrode configurations of the prior art.

In a preferred embodiment of the present invention, a non-concentric-cylindrical electrode FAIMS system is deployed. This system does not have detection limitations of conventional concentric cylindrical FAIMS and may be generally referred to as a non-focusing, wide-spectrum FAIMS system. Preferred systems of the invention feature good diffusion of ions throughout the analytical gap.

The present invention enables innovative techniques for separating and identifying biomaterials based on ion mobility characteristics of their biomarkers. Furthermore, embodiments of the invention can simultaneously filter and detect both positive and negative ions of an ion species, unlike the single mode at a time of the prior art. As a result, a fast and accurate biomarker analysis system may be provided in a compact and field-portable package.

We have achieved a unique combination of high sensitivity (ppb-ppt), simple and rugged construction, small size, mass producibility, and relatively low cost. It is also possible in practice of the invention to provide new information which is not ordinarily made available by other techniques of chemical analysis. Therefore systems

of the invention may be used alone or in combination with other analytical equipment with increased likelihood of accurate identification of chemical compounds, even at trace levels, and even for complex mixtures that heretofore have been difficult to resolve.

5

BRIEF DESCRIPTION OF THE DRAWINGS:

The foregoing and other objects, features and advantages of the invention will be apparent from the following more particular description of preferred embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating the principles of the invention.

Fig. 1 is a schematic of a plate-type wide-spectrum differential mobility spectrometer in practice of the invention.

Figure 2 shows overlapping prior art TOF-IMS spectra for m-Xylene and p-Xylene isomers.

Figure 3 shows resolved DMS spectra for m-Xylene and p-Xylene.

Figure 4 shows positive ion spectra for different concentrations of methyl salycilate.

Figure 5 shows concentration dependence of the invention to methyl salycilate for both positive and negative ion spectra.

Figure 6 shows response for prior art FID and DMS of the invention as a function of compound concentration for a homologous ketone mixture.

Figure 7 shows spectra for a DMS embodiment of the invention with a GC front-end.

Figure 8 shows spectra for the GC-DMS where the chromatographic runtime has been decreased leading to co-eluting species and showing that a practice of the invention is able to resolve the co-eluted species.

Figure 9 shows comparison of prior art FID and a DMS embodiment of the invention for reproducibility for a homologous alcohol mixture.

Figure 10 is a schematic of an embodiment of the invention with a pyrolysis front-end.

Figure 11 shows positive and negative ion spectra for PA in practice of the invention.

Figure 12 shows positive and negative ion spectra for DPA in practice of the invention.

Figure 13 shows positive and negative ion spectra for pyridine in practice of the invention

Figure 14 shows spectra with putrescine and cadaverine resolved from one another in practice of the invention.

Figure 15 shows background spectra with no sample present on a SPME fiber.

Figure 16 shows spectra obtained from subject #1.

Figure 17 shows spectra obtained from subject #2.

Figure 18 shows spectra generated for biomarkers for bacillus spore pyrolysis in practice of the invention.

Figure 19 shows positive ion spectra for urine headspace detected in practice of the invention.

DETAILED DESCRIPTION OF THE INVENTION

A description of preferred embodiments of the invention follows.

While this invention has been particularly shown and described with references to preferred embodiments thereof, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the scope of the invention encompassed by the appended claims.

In an illustrative embodiment of the invention, a preferred planar, wide-spectrum differential mobility spectrometer is employed as a tunable ion filter, operable at or around atmospheric pressure. Figure 1 is a schematic of a plate-type high field differential ion mobility spectrometer (DMS) in practice of the invention having planar electrodes on insulated substrates. The DMS is an improved analytical system which employs FAIMS high-field RF asymmetric ion filter techniques.

As a gas sample S is introduced into spectrometer 10 it is ionized by ionization source 12 and the ions and neutral molecules (general referenced as 14) are transported along flow path 16 through plates 20, 22 of an ion filter 24 and towards detector region 30. The ions are transported by a transit mechanism, such as a carrier gas flow 18.

The ion filter 20 is electronically tunable and an ion species 14'(+,-), is allowed to pass through the filter. Species 14' is selected by adjusting the high field generated between filter electrodes 20, 22. This tuning is done by adjusting the compensation of the field, which correlates to species specific differences in high field mobility of the ions in the field. Unwanted ions, i.e., those for whom the compensation is inadequate (i.e., "uncompensated" ions) are scattered toward and collide with the ion filter electrodes and are intentionally neutralized by such contact.

Depending on the electric field conditions, e.g. RF field strength, duty cycle, compensation, flow rate, ion species are selected and permitted to pass through the ion filter region for detection. An ion species is then identified based on its high field mobility signature, which is determined based on knowledge of the conditions used to generate the spectra. Field strength can be adjusted as needed, and ranges from around 5,000 to about 40,000 V/cm in preferred embodiments, in an analytical gap of about 0.5mm. In one embodiment, an asymmetric RF voltage applied to the ion separator electrodes was in the range of about 900 to about 1.5 kV (high field condition), and a low voltage of about -400 to -500 V (low field condition), at about 1-3 MHz, wherein the high frequency operated at approximately 30% duty cycle. Other embodiments are also within the scope of the invention.

In the embodiment shown, as the passed ions impact detector electrodes 32, 34 of detector 30 they transfer their charge to the electrode and the resulting current is read by an electrometer. This detection data is correlated with field conditions (voltage, frequency, duty cycle etc.) and is compared to stored data to enable identification of the detected ion species.

In practice of the invention, ion species are filtered based on mobility differences and not based on their polarity. Therefore in a preferred practice of the invention, all ions of an ion species will be passed on for detection, whether positive or negative ions, and which may be detected simultaneously. Accordingly detector electrodes are biased so that one attracts the positive and the other attracts the negative ions. Thus, in an embodiment of such arrangement both "positive mode" and "negative mode" ions of a species are detected simultaneously. In this embodiment, having both modes from a single detection provides a more unique signature for the detected ion species and therefore increases the potential accuracy of species identification of the invention.

To illustrate the advantages of the method and apparatus of the invention, compounds that are extremely difficult to resolve in time-of-flight ion mobility spectrometry (TOF-IMS) are shown to be easily resolved herein. TOF-IMS is a highly sensitive, quantitative method for organic compound detection. It has been used for detection of chemical warfare agents, illicit drugs and explosives, and unlike mass spectrometry, it operates like the present invention at atmospheric pressure, eliminating the need for vacuum tight seals and power consuming vacuum pumps.

However, the TOF-IMS operates with low strength electric fields where the mobility of an ion is essentially constant with electric field strength, while the present invention operates in periodic high fields and filters based on the non-linear mobility dependence of ions on the high strength fields. Thus the invention can provide more and different structural information about ion species that further enables accurate species detection and identification. Further comparison with TOF-IMS is instructive.

It will be understood that mixed xylenes are the second-most-important aromatic product for chemical manufacturing around the world, ranking behind benzene and ahead of toluene. Of the three isomers (ortho-, meta- and para-xylene) p-xylene is the most widely used isomer in the manufacture of polymeric materials. Separation of these isomers is generally challenging with most analytical instruments. Since these isomers have the same molecular weight they cannot be resolved in a mass spectrometer.

In conventional TOF-IMS these compounds have virtually overlapping peaks, as shown in Figure 2. While these compounds can be resolved in a gas chromatograph (GC), this typically takes more than 20 minutes. Meanwhile devices in practice of the invention enable excellent resolution of the para and meta xylenes in under one second. Figure 3 shows these compounds clearly resolved in practice of the invention, notwithstanding such surprisingly rapid performance.

Figures 4 and 5 show the response and concentration dependence in practice of the invention for methyl salycilate, a chemical warfare agent stimulant. Figure 4 shows positive ion spectra for different concentrations of methyl salycilate. Figure 5 shows concentration dependence of the system to methyl salycilate for both positive and negative ions. Samples with concentrations of methyl salycilate down to about 45 parts-per-trillion are readily detectable in this device. The methyl salycilate compound produces both positively and negatively charged ions which exhibit similar concentration dependences. The apparatus of the invention is able to simultaneously detect both ion responses within the same analytical run. Producing simultaneous positive and negative ion species information improves compound identification at reduced detection times.

To further characterize the spectrometer 10 of the invention, it was interfaced to a GC and used as a chromatographic detector. The system performance was compared with that of the most widely used GC detector: the Flame Ionization Detector (FID). Figure 6 shows a comparison of the concentration dependence and detection limits measured by the FID and by a DMS practice of the invention for a homologous series of

ketones. Response is shown as a function of compound concentration for the ketone mixture, with average detection limits of the invention approximately an order of magnitude better than those of the FID. Thus the average FID detection limit is 2E-10g, while a preferred wide DMS system of the invention has a detection limit of 2E-11g.

Similarly to a mass spectrometer, the ion information provided by the invention offers a second dimension of information to a GC chromatogram and the ability to enhance compound identification. Figure 7 shows spectra according to a GC-DMS embodiment of the invention, with the part shown as a chromatogram (right frame) being typical of what is seen from a FID. In practice of the invention, the chromatogram is the sum of the peak intensities for the product ions created. The associated two-dimensional plot (left frame) of ion intensity (indicated by gradient) versus scanned compensation voltage provides a means of fingerprinting the compounds eluted from the GC. Therefore practice of the invention provides three levels of information: retention time, compensation voltage, and ion intensity, all shown on the spectra of Figure 7. Furthermore, spectra may be obtained simultaneously for positive and negative ions, eliminating the need of serial analysis under possibly changing instrumental conditions, as required with other equipment.

The wealth of information provided in practice of the invention can eliminate the need for external calibration through standards. In addition, if chromatographic conditions (i.e. fast temperature ramps to reduce analysis time) result in co-eluted peaks, the present invention can resolve these peaks and elucidate compound information, as shown in Figure 8, which is an example where GC runtime was decreased to generate co-eluting species that are resolve in the DMS spectra. In this way, a fast GC can be used while maintaining the required compound resolution. Furthermore, the reproducibility of the present invention compares very well to that of the FID as shown in Figure 9. (Figure 9 shows a comparison of FID and DMS reproducibility for a homologous alcohol mixture.)

Use of the present invention can improve the speed and reliability of identification of materials whether in the laboratory or in the field. This follows because of the inherent speed, reliability and sensitivity of the invention, the additional unique information provided by the invention, and its compact size and portability.

Turning to an illustrative embodiment of the invention, shown in Figure 10, method and apparatus are provided for detection of spore-forming bacteria based on signatures of biomarkers isolated in an illustrative pyrolysis-wide-spectrum DMS system of the invention.

In this embodiment, system 100 includes a sampler or other particle collector 102 which delivers liquid or solid sample to a pyrolizer 104 (such as a commercially available pyrolyzer from CDS Analytical) which has an output coupled to the flow path 106 of the DMS analyzer 110. The flow path structure (sometime referred to as a drift tube) has an inlet 112 for receipt of the pyrolized sample output from the pyrolizer carried by carrier gas 114. The pyrolate in transferred from the pyrolizer to the DMS through a sealed and heated interface. During sample loading on the probe, the pyrolysis chamber is purged while a stream of clean N2 is diverted into the DMS. During pyrolysis, the flows are diverted through a 6-port valve into the analyzer to assist introduction of the pyrolate.

In one example, the pyrolyzer heated samples from room temperature to 1400 C at rates from 10 - 20 °C /msec. The controlled temperature ramping enables selective desorption of compounds from the probe, therefore enhancing resolution and signal-to-noise of the apparatus. A drying function evaporates and vents the solvent out a purge vent resulting in sample concentration and prevention of the solvent from entering the DMS analyzer 110. A probe cleaning function, flash-heats and desorbs left-over sample between analyses.

The pyrolate is carried by the carrier gas into the ionization chamber 120 where source 122 ionizes the sample. The ions ("+", "-") are carrier by the carrier gas into the filter 124 between filter electrodes 126, 128. In a preferred practice of the invention, an

asymmetric high RF field is generated between the filter electrodes, with applied DC compensation, under control of controller/driver 130. Ion species are passed to the detector 132 based on compensated field conditions and mobility difference for the species. As the compensation is scanned, a spectrum can be recorded for the sample. Detector 132 includes electrodes 134, 136, which enable detection of positive and negative modes for each species.

In a preferred embodiment, electrodes 124, 134 are formed on an insulating substrate 140 and face electrodes 126, 136 formed on substrate 142. The substrates are mated to fix the distance between the electrodes and defining the analytical gap G between the electrodes (preferably ~0.5mm). The asymmetric field is generated between these electrodes transverse to the analytical gap and the ions are flowed in the gap through the field.

BIO DETECTION EXAMPLE 1:

In one practice of the invention of Figure 10, biomarkers that are representative of a general class of bacterial spores or sporulating cells was analyzed. Detection of dipicolinic acid (DPA) as a biomarker was demonstrated. We also show the intermediate pyrolysis product of DPA, namely picolinic (2-pyridinecarboxylic) acid (PA), which is a highly specific biomarker for sporulating microorganisms. We further show detection of pyridine, generated by pyrolysis-induced decomposition of the DPA in the pyrolized sample, and as a further biomarker for the sporulating material.

Positive and negative spectra for picolinic acid are shown in Figure 11 and for DPA are shown in Figure 12. The spectra were obtained from solid samples that were pyrolyzed sequentially in the same operating conditions. The picolinic acid was pyrolyzed through a temperature excursion of 130 to 300 °C at a rate of 20,000 °C/s, the interface temperature was held at 130 °C. Dipicolinic acid was pyrolyzed from 145 to 400 °C at 20,000 °C/s, and the interface temperature was held at 145 °C.

Both PA and DPA produce positive and negative ion peaks that can be used for identification and which were simultaneously detected in practice of the invention. It will be appreciated that it is further beneficial that these positive and negative spectra, having being generated and detected at the same time from the same sample in the same conditions, can be reliably associated with each other and the detected species in practice of the invention. The result is a more robust and more reliable process of biomarker detection and identification.

In practice of this embodiment, the RF field was set at about 40,000 V/cm and the compensation voltage was scanned to generate a profile of detected sample.

As shown in Figure 11, a detection peak for PA was generated at -1.72 v compensation in the positive ion detection mode and -3.65 volt compensation in the negative ion detection mode, which is a signature for this sample. The DPA was detected at -2.52 v compensation in the positive mode and 4.-3 volt compensation in the negative mode, which also is a signature.

It is further noted that the DPA produces a secondary positive ion peak at -0.27 volts, further differentiating its signature. The peak width at half height averages 1.4 V. Thus it will be understood that bio-compound identification can be relatively straightforward in practice of the invention.

Furthermore, it is known that pyrolysis is capable of fully decarboxylating DPA to pyridine. Ideally, controlled and more gradual pyrolysis conditions will lead to loss of only one carboxylic acid group to generate PA, enabling specific identification of the DPA source as bacterial spores. Nevertheless, spectra for pyridine were generated.

As shown in Figure 13, positive and negative ion spectra for pyridine were obtained, done in the same conditions as those for the DPA/PA analysis. It will be noted that pyridine does not produce negative ions and therefore has a relatively flat negative spectra. The absence of a negative ion peak enables one to conclude that the pyrolysis conditions employed are mild enough to prevent full decarboxylation and that pyridine can be differentially detected thereby according to the positive spectra as shown.

It will be further appreciated that in embodiments of the invention detections are made and then the identification process typically involves comparison against a lookup table of stored detection data. Thus a practice of the invention not only results in detection of a biomarker but also results in indication of the bio-threat with which the biomarker is associated. For example, if spores were in a sample, the above detection results would be obtained and would be compared against a store of related detection data. Upon a positive match, an identification announcement would be made.

Preferably the apparatus of the invention includes an on-board pyrolizer and wide-spectrum DMS analyzer, wherein collected samples are pyrolized and then resulting gas sample is automatically transported to the analyzer and then detected for evaluation of presence of potential bio-threat in the sample based on high field ion mobility chemical signatures. In a further embodiment, the pyrolizer and DMS device may be made using MEMS technology in a single package. The sample collector may also be on-board.

In a further embodiment of the present invention, a sample is identified by a multi-stage FAIMS analysis, wherein a first stage filters a sample by particle size and defines a narrowed sample set, and in a second stage this sample is pyrolized and then analyzed based on high field ion mobility as discussed. Results of the first and second stage are correlated with known standards to identify the compounds in the sample.

BIO DETECTION EXAMPLE 2:

The present invention has application in the biomedical field as well detection of bio-threats. As an example, it is widely known that the presence of biogenic amines in human body fluids such as urine, saliva, and blood, may reveal or suggest pathological conditions or dysfunctions. For example, elevated levels of particular biogenic amines in urine may indicate the presence of cancer. Chemical changes in the living system or degradation processes of cells after death are accompanied by the formation of molecular byproducts. These processes include the breaking down of peptides and DNA

strands to smaller components, and changes in amino acids that lead to the formation of amines. One of the processes of particular interest is the breakdown of amino acids and the production of diamines and polyamines.

Furthermore it is known that decarboxylation of ornithine and lysine produces putrescine and cadaverine respectively. An atmospheric pressure ionization method of the invention is particularly suited for the detection of these markers, such as biogenic amines, since they tend to have either high proton affinity and form stable positive ions or high electro-negativity and readily form negative ions.

Figure 14 shows spectra detected according to a food-quality embodiment of the invention for a mixture containing both putrescine and cadaverine. The putrescine peak at about -30 volt compensation is well separated from the cadaverine peak at -29 volts, and which are separate from the detected n-Nonylamine. Based on these results, it will be appreciated that a food-quality detector of the invention can be used to evaluate the quality of a food sample, such as meat, based on the detected presence and intensity of these bio-markers.

BIO DETECTION EXAMPLE 3:

Another application of the invention is in breath analysis. The human breath contains over 400 organic compounds at concentrations typically in the parts-per-million (ppm) to parts-per-billion (ppb) range. Only a slender barrier, the pulmonary alveolar membrane, separates the air in the alveoli of the lung from the blood flowing in the capillaries. This membrane allows volatile organic compounds to easily diffuse from the blood into the breath. Moreover, the concentration of these compounds in the breath can be correlated to their concentration in the blood, as noted through the widespread use and acceptance of a breath analyzer to determine alcohol consumption.

Through systematic studies, concentrations of particular compounds have been correlated with specific diseases or impairments in metabolic pathways. However, while these studies are encouraging, there are still a number of complicating factors which

have limited wide spread adoption of breath analysis for medical diagnosis. These include: the complexity of current breath analysis systems, their high cost, amount of correlation between the data and disease, and the complexity of data analysis due to interferences and moisture.

In practice of the present invention we provide a non-invasive breath analysis system. In these experiments, sample collection involved collecting a breath sample directly onto a solid phase micro-extraction (SPME) fiber assembly. The SPME fiber was placed in proximity to the mouth of the subject and the sample collected for two minutes. The SPME assembly was then inserted into a GC injector port which was held at 120C and desorbed the sample from the fiber into the GC column. The present wide-spectrum DMS was attached at the detector port of a GC.

A background baseline spectra without sample on the SPME fiber is shown in Figure 15. Spectra from subject #1, Figure 16, and subject #2, Figure 17, are very similar except for the peak at a compensation of about -3 volts for specimen #2. Using the GC alone, without the benefit of the present invention, the presence of these different compounds would not be evident. The resultant GC-DMS plots shows the chromatographic retention time on the y-axis and the compensation voltage plotted on the x-axis and shows the value of the detector in providing additional information to simplify and assist in the analysis of a human breath sample.

BIO DETECTION EXAMPLE 4:

It will be appreciated that the present system enables detection of biomarkers for a wide range of bacillus. As an additional demonstration, shown in Figure 18, spectra for biomarkers from pyrolyzed *B. subtilis* were identified. Spectral scans for pyrolized water sample are shown in A, 40,000 spores pyrolized are shown in B, and 120,000 spores pyrolized are shown in C. A person skilled in the art will recognize from this data that biomarkers at 1, 3 and 4 correlate with the presence of spores, with amplitude corresponding to concentration.

Thus it is clear that the present innovation can provide a compact and portable detection system for detection of biological materials, whether they are introduced directly from the air or via a swab, followed by pyrolization.

BIO DETECTION EXAMPLE 5:

Embodiments of the present invention also include use of DMS for medical diagnostics, which includes examination of metabolites in a sample (such as taken as breath exhalation, blood, sweat, other bodily fluid, etc.) as an indicator of health status. This can also include indication of presence or absence of a virus, disease, pathogen, or the like.

In one experiment mouse urine samples were tested using DMS as shown in Figure 19 showing positive DMS spectra. In the figure, a large carrier gas (N2) peak (~ 3 a.u.) is seen at 0 Voltage Compensation (Vc), while urine headspace (vapor) detection spectra is seen just to the left (< 0 Vc).

In this demonstration, urine sample headspace vapor from three different individual B6-H-2b male mice was analyzed. The DMS spectra indicated the urine samples were similar to each other but different from two control monomolecular odorants (isovaleric acid and isoamylacetate). A small number of sample preparation permutations were tested to identify conditions that yielded the most volatiles as indicated by intensity in the DMS spectra. Addition of: 1.) water (to increase volume of urine sample), 2.) salt (0.2 mg/µl), and 3.) heat (37 C), all yielded more volatiles.

In view of the foregoing, it will now be understood that we are able to demonstrate use of DMS as a biological evaluation tool for medical diagnostics, such as for urinalysis. It is also noted that such testing does not require fresh liquid samples. While freezing and thawing of such samples reduces the amount of volatiles, still detection can proceed. In practice of the invention, a data base of urine and analyte samples can be determined and stored. \

Now a lookup function enables identification of ion species detected in urine. This experiment is illustrative of establishing baseline upon which a specific detector

can be established. For example, detection of such analytes as pronase, (NH4)2SO4, KH2PO4-H2O, CK2O3, or even NaCl can be detected.

It will now be appreciated that the present invention benefits from the realization that an adaptation of our wide-spectrum DMS scales down well, even allowing miniaturization of the analytical cell. Surprisingly, this scaling has been achieved while preserving sensitivity and resolution. Furthermore, the smaller units in practice of the invention have reduced volume and reduced support requirements, with flow volumes and power requirements reduced accordingly. A smaller apparatus also reduces internal ion travel distances and travel times, which results in faster detection processes.

Novel methods and apparatus of the invention enable rapid detection and identification of compounds, even of compounds that cannot be or are difficult to resolve by other analytical techniques. Embodiments of our compact spectrometer offer a unique combination of high sensitivity (ppb-ppt), new analytical information, simple and rugged construction, small size and mass producibility.

It will therefore be appreciated that we provide a novel method and apparatus for chemical and biological sensing applications. The invention fills an unmet market need, providing spectrometer capabilities and extremely high sensitivity.

Various modifications of the embodiments set forth above are within the spirit and scope of the present invention. For example, other shapes or configurations of structures, electrodes, spacers, and substrates, among others, are within the spirit and scope of the present invention. The specific construction techniques set forth above are provided by way of illustration and not by way of limitation of the scope of the invention. The terms detector, spectrometer and sensor may be used interchangeably for purposes of this disclosure within the spirit and scope of the present invention. The terms drift tube, flow channel and flow path may be used interchangeably and remain within the spirit and scope of the invention. The terms contact pad and bonding pad likewise may be used interchangeably within the spirit of the invention. The terms upper lower inner and outer are relative, are used by way of

illustration and not by way of limitation.

It will be further appreciated that the present invention is operable with gas and liquid samples, even though for convenience the illustrative examples above refer to samples in a gas flow. The scope of these and other embodiments is limited only as set forth in the following claims.